

STABILIZATION OF LOCOMOTOR ACTIVITY RHYTHMS OF PIGLETS
INTRODUCED INTO A NEW ENVIRONMENT

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16. Abstract By means of photocells, it has been shown that the general activity of groups of piglets follows a <u>nycthemeral</u> rhythm with two diurnal peaks, a morning peak and a larger afternoon peak. The animals were moved to a new environment and a systematic study of the changes in the parameters of this rhythm was formed based on the following measurements: rhythm period computed by an autocorrelation function, amount of diurnal activity compared to total daily activity, statistical indices of activity distribution based on the 25 and 75 percentiles and the median of the hourly activity percentage cumulative curve. The period of adjustment to the new environment was characterized by a progressive decrease in the diurnal activity (Fig. 3) and an increase in its dispersion (Fig. 4) during the first three days. There was no modification in the rhythm itself.			
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STABILIZATION OF LOCOMOTOR ACTIVITY RHYTHMS OF PIGLETS INTRODUCED INTO A NEW ENVIRONMENT

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Introduction

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In previous experiments we used an actographic setup involving the occultation by the animals, during their movements, of beams of infrared light projected onto photo-electric cells (Dantzer and Mailhe, 1972a) to reveal a nycthemeral rhythm of activity in group-bred piglets (Dantzer and Mailhe, 1972b).

The use of these rhythms in longitudinal pharmacological experiments presupposes their definite stability prior to any treatment. The usual step taken for this purpose consists in allowing a given time -- on the order of a few days to a few weeks -- to elapse after the animals have been introduced into the new environment, prior to the start of measurements. This is an extremely widespread procedure, and recently Grant et al. (1972) have drawn attention to this problem by demonstrating, on the basis of water-drinking behavior, that rats require three weeks to reach stable behavior.

To obtain specific objective results on this phenomenon, we have made a systematic estimate of the time interval required for stabilization of the rhythm of locomotor activity in several groups of piglets transferred to a new sty.

The results will be given below.

Techniques

- 1) Animals used and breeding conditions

* Numbers in margins indicate pagination in the foreign text.

The animals used were piglets two and one-half months old at the beginning of the experiment and weighing an average of about 20 kg. Four successive groups were studied; each of these contained two castrated males and two females, with the exception of one group consisting of four females. The /497 animals in each group were from two different litters, with one animal of each sex from each litter.

The piglets were weaned in the initial sty when they were about 7-8 weeks old. Additional food in granulated form was distributed from the third week on, and the animals were fed in the morning, usually between 7:00 and 8:00.

The new environments had an available area of 6 m² with a rest area 2 m. on a side and a corridor with slatted flooring, with one meter out of every two without separations. Here the piglets obtained food ad libitum in the form of dry flour, water being dispensed by two automatic drinking troughs. The sty was cleaned in mid-morning every day of the week except Sunday.

The sty was lighted artificially from 6 a.m. to 8 p.m. and its temperature was kept at a level close to 20°C by a ventilation and heating system.

2) Actographic setup

Four projectors with infrared filters, two along the width of the sty at an interval of 70 m and two along the length at an interval of 1 m, transmitted a beam of light onto symmetrically arranged photoelectric cells delimiting nine equal rectangles with an area of 0.70 m². The cells and the projectors were attached 35 cm above the ground, so that the beam could be intercepted only by an animal which was standing

or at least sitting back on its hind legs. Any interruption of the beam provoked a single pulse no matter how long the subject remained in front of the cell. The pulses obtained were totaled on a printing counter; this was connected with a clock used to program the time interval during which the pulses were counted. This interval was set at one-quarter hour.

3) Processing of results

We chose to perform a longitudinal study in which the animals are considered to be their own control, with the basic focus being variations in the activity parameters during the first 20 days after the animals were placed in the sty.

In tabulating the results, a day was defined as a 24-hour time period occurring from minute to minute. Recordings were begun 12-16 hours after the piglets were placed in the sty. Thus they do not allow for the initial locomotor reactions of the animals basically pertaining to the establishment of social relationships. However, this is an extremely early and short-term phenomenon, lasting only a few hours, so that it would be undetectable in experiments based on a 24-hour scale.

On the basis of the variable used, the numbers of pulses totaled per quarter hour, the period of the rhythm was estimated by analysis of the correlogram, with the correlation of order k between two values u_i and u_{i+k} with time lags k being calculated by the formula:

$$r_k = \frac{\frac{1}{n-k} \sum_{i=1}^{n-k} (u_i - \bar{u})(u_{i+k} - \bar{u})}{\frac{1}{n} \sum_{i=1}^n (u_i - \bar{u})^2}$$

with $H = \frac{1}{n} \sum_{i=1}^n m_i$ (Valleron and Dolais, 1971), n being the number of terms in the series studied.

However, application of the Shannon theorem makes it necessary to set a limit on the usable order k, so that the correlation will keep a statistical value representative of the sum total of data ($k_{\text{limit}} = \frac{2n}{10}$). Consequently a 24-hour rhythm can be /498 detected only if the analysis deals with at least six days.

On the basis of previous results (Dantzer and Mailhe, 1972b) indicating the predominance of diurnal activity and its distribution into two characteristic peaks, the following measurements were used to evaluate the time distribution of daily activity:

-- the level of activity during the illuminated period (diurnal activity);

-- the estimated median and 25 and 75 percentiles from the cumulative curve of variations in hourly activity over time, expressed as a percentage of total daily activity. The latter parameters were used to compute two indices:

1. A dispersion index I_D , represented by the distance, in hours, between the 25 percentile and the 75 percentile;

2. A relative index I_R , representing the ratio of the time between the median and the 25 percentile to the time between the 75 percentile and the median. Due to the position of the percentiles, this relative index permitted an evaluation of the differences between the morning peak and the evening peak.

3. The correlograms and the various parameters considered were calculated on a computer (CII 10070).

Results

1. Analysis of correlograms

The correlograms calculated on days one through six, seven through 12 and 13 through 20 show that, as a general rule, the activity at a given instant correlates at a value of 0.30 to 0.40 with the activity observed 94 to 98 quarter-hours later, which indicates the presence of a subjacent period rhythm occurring between 11:30 and 12:30 p.m. Fig. 1 gives an example of a correlogram of this type. Sometimes there is a second, less significant, negative correlation peak at the 12th hour (Fig. 2), which indicates that there is marked opposition between high diurnal activity and extremely low nocturnal activity. Within each group, the shape of the correlograms does not reveal systematic changes in the location or value of the correlation peaks between the different times of measurement.

2. Variations in intensity of activity

Fig. 3 shows the variations over time of the total activity and the diurnal activity of the sum total of groups considered. There is a highly significant relationship between these two variables ($r = 0.97$ to 70 ddl), while the correlation between total activity and nocturnal activity is much less marked ($r = 0.64$) and is still weaker between diurnal activity and nocturnal activity ($r = 0.45$). The overall activity, which was extremely high the first day, decreased rapidly and subsequently stabilized, with the bending point occurring between the third and fourth days. From day 4 to day 6, this overall activity was reduced to an average of 4139 pulses, as against 5821 during the first three days ($F_{64} = 6$; $P < 0.025$); subsequently it showed no significant variations. The apparent drop in activity beginning on day 18 is completely due to the lack of results from the most active group due to the malfunctioning of one photocell. This failure did not affect the other activity distribution parameters for this group.

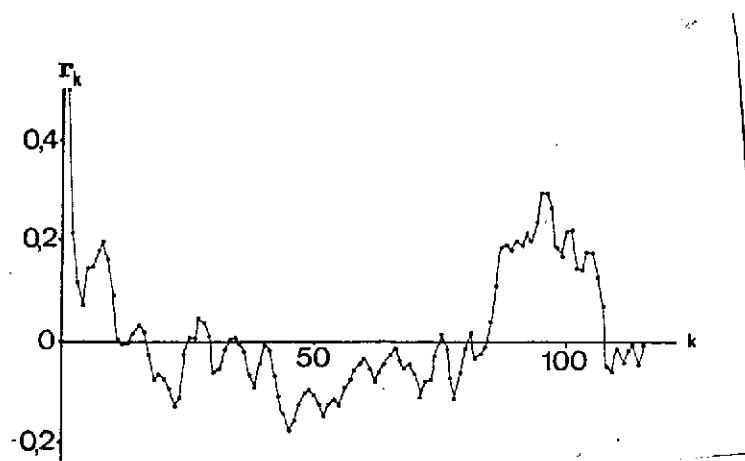


Fig. 1. Correlogram calculated from the pulses obtained from group 3 during days 1 through 6. Ordinates: correlation r_k as a function of the order k representing the number of quarter-hours between the data used for calculation.

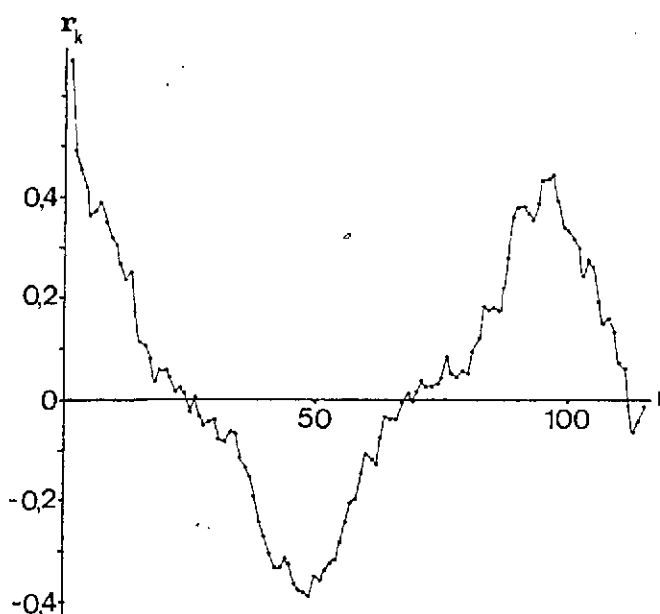


Fig. 2. Correlogram calculated from the pulses obtained from group 2 during days 1 through 6. Notation same as in Fig. 1.

3. Analysis of statistical parameters of distribution of daily activity /501

The dispersion index (Fig. 4) reached its lowest value during the first two days (7.50). It then increased, since during the period from day 3 to day 6 and that from day 7 to day 13, it reached an average value of 8.27 ($F^1_{64} = 3.80$; $P < 0.10$). It increased again between the 14th and 20th days; thus an average of 8.76 hours were required

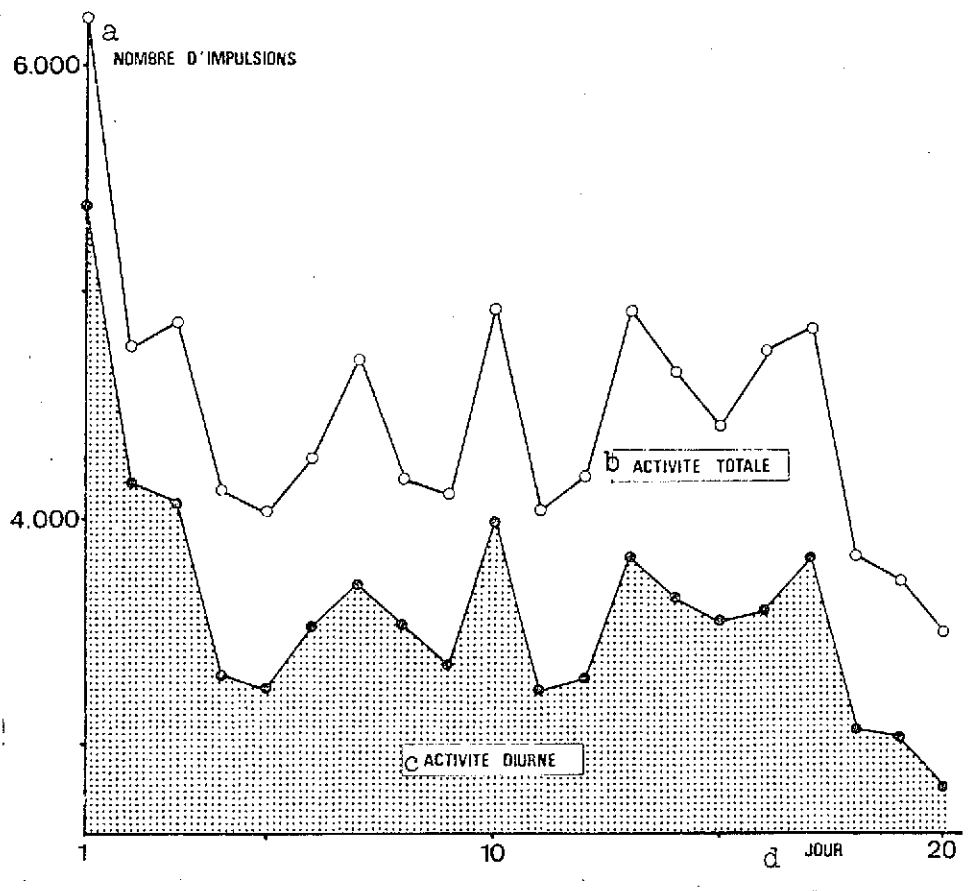


Fig. 3. Variations in total number of pulses and number of pulses collected during the diurnal phase of the cycle. Each point represents the computed average of the sum total of results.

Key: a. number of pulses
b. total activity
c. diurnal activity
d. day

for the activity to increase from the 25 percentile to the 75 percentile ($F_{64}^1 = 3.70$; $P < 0.10$). The wide variability of this index limits its use,

however. This is not the case with the relative index (Fig. 4), which increased significantly during the first seven days ($F_{19}^1 = 17.22$; $P < 0.001$), before it stabilized, beginning on

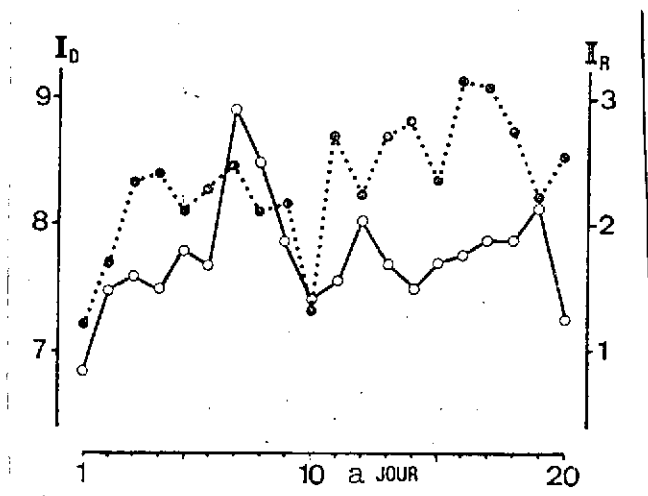


Fig. 4. Variations of dispersion index I_D (●---●) and relative index I_R (○---○). Each point represents the computed average of the sum total of results.

Key: a. day

-- a decrease in the time required for the change from the 25 percentile to the 75 percentile;

-- a low relative index, which required approximately eight days to reach a high stable value;

...these phenomena being more or less marked within each individual group.

Discussion and conclusion

Depending on the variable used, the time lapse necessary for stabilization of the rhythm of activity may be zero (period of rhythm), on the order of three days (number of pulses

the ninth day, around an average value of 1.70 ($F_{27}^1 = 0.304$; N.S.). This manifested the characteristic predominance of the evening peak over the morning peak.

To summarize, the motor activity collected during the first days after the animals were introduced into the new breeding sites showed the following differences from the "stabilized" activity:

-- hyperactivity, primarily during the diurnal phase of the nycthemeral cycle, which took approximately three days to dissipate;

collected, dispersion index), or approximately eight days (relative index). This apparent disparity may be attributed to the fact that each variable represents a different level of analysis:

1. The period itself is the fundamental characteristic of the rhythm. There are two possible explanations for its apparent lack of change. The first, statistical in nature, arises from the necessity of dealing with six consecutive days in analysis of the correlograms, which could obscure extremely early disturbances, at least in part. The second involves the abundance of synchronizers (Aschoff, 1960) in the environment used (light, temperature, movements of personnel, etc).

2. The number of pulses collected constitutes another /502 dimension of the rhythm: there are three possible causes for the initial hyperactivity, which was especially marked during the diurnal phase of the cycle:

-- first, fighting between animals due to their inter-mixing; however, observation has shown that this did not occur very frequently during the first day of recording (the animals having been placed in the environment the morning of the day before) and that it was completely lacking on subsequent days;

-- second, exploration of the new environment, but with the same restrictions as above;

-- finally, hyper-reactivity of the animals to the normal human environment of the sty, since each intrusion resulted in a "cry of alarm" from one of the animals at rest (Signoret, 1969), provoking locomotor reactions of flight within the group. These reactions decreased and usually disappeared after a few days in the sty.

3. There were still possibilities of variation even after the diurnal activity had become stabilized, since there could

be different distributions corresponding to a given level of activity. The dispersion index (or interquartile gap) is equivalent to a variance and is valid only as long as the distribution is uniform. Thus it does not allow for the bimodal nature of the distribution of diurnal activity demonstrated in "stabilized" animals, and characterized by two peaks, one morning peak and a more significant evening peak, with a minimum of activity at about 1:00 to 2:00 p.m. (Dantzer and Mailhe, 1972b). This is where all the value of the relative index lies, since by figuring the ratio of the time between the 75 percentile (occurring at about 5:00 p.m.) and the median (approximately 2:00 p.m.) to the time between the median and the 25 percentile (in the vicinity of 9:00 a.m.), it can be used to estimate the difference between the evening peak and the morning peak. The latter requires eight days to stabilize, beginning at a value close to 1 to reach a final value of approximately 1.80.

It thus appears that increasingly thorough analysis will reveal increasing complexity in the stabilization process. However, it seems difficult to make any further evaluation of the locomotor activity itself, except perhaps for breaking it down into its component parts, since a single activity can serve as the basis for feeding behavior, an aggressive reaction toward a congener, or explorative behavior (even without any attempt at exhaustive analysis), while the lack of activity does not necessarily indicate sleep. The distribution of these components probably follows a line of development comparable to that of the activity on a qualitative level, but not necessarily on a quantitative level.

The results obtained by Grant et al. (1972) on the amounts of water consumed by Lister rats indicate the possibility that there may be stabilization phases on the order of a few weeks. These investigators state that they obtained comparable results

for the activity and the volume of feces and urine, but these results were not presented. Aside from the difference in species, the degree of contrast between the initial situation and the new environmental conditions is a factor which is difficult to evaluate and whose importance cannot be negligible. Unfortunately, in the absence of other research along these lines, /503 it is hardly possible to speculate or generalize on data of this type, which are still highly incomplete. However, since this problem is a daily concern for biologists performing longitudinal tests on groups of animals, there should be ample opportunity for fruitful development in this field.

In conclusion, the examination of progressive changes in the locomotor activity rhythms of groups of piglets placed in new environments has made it possible to show that approximately eight days are required for the onset of stable rhythmic characteristics. This provides objective support for the complex problem of determining the period of adjustment to experimental conditions in longitudinal studies.

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